ANAEROBIC CONVERSION OF WATER HYACINTH TO BIOFUEL USING ALKALINE WASTE

Synopsis
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INTRODUCTION

Global energy crisis and environmental change have recently become the most concerning issues. These problems are primarily due to the rapid industrialization, mass development, and expanded use of petroleum products (Wirth et. al., 2018). The total population by 2050 has been predicted to be 1.5 times the current population. Never before the need and challenge for sustainable production methods for food and energy was larger than in this century (Sajjadi et. al., 2020). Many nations are still using unconventional fuels as an important source of energy which has caused catastrophic land degradation and environmental change due to the depletion of greenhouse gas (Pourkarimi et. al., 2018). However, petroleum products around the world are used for power generation, the combustion process of these fuels results in various hazardous effects causing pollution. During the fuel consumption, several compounds like ground-level ozone, nitrogen oxides (NOx), volatile organic compounds (VOCs), carbon monoxide (CO), particulate matter (PM), sulphur dioxide (SO_2), carbon dioxide (CO_2), and some other heavy metals are commonly observed (Saravanan et. al., 2018).

The unexpected expansion of unspecified fuel costs and uncertainties associated with its accessibility has recently been considered an important drive for alternative clean and lowpriced sustainable energy (Machineni et. al., 2019). Environmental concerns regarding fuel consumption and its energy sources draw much attention to finding stable and pure energy assets (Pourkarimi et. al., 2018). Renewable energy efficiency may satisfy global energy demand, but at the same time are important barriers to reducing greenhouse gases emission. There are many ways to produce fuel from renewable resources; however, their commercial productions are still a limiting factor (Joshi et. al., 2017). Biofuel drives significant considerations for being a source of energy that is environmentally friendly and non-toxic, biodegradable, and does not have any negative effect on climate and the environment (Singh et. al., 2018). The advantages of biofuels over petroleum fuels are (Ninawe et. al., 2017).

(a) more environment friendly.

(b) can be successfully extracted from biomass,

(c) its combustion depends on the carbon-dioxide cycle,

(d) are sustainable because they can be biodegraded,

Biofuel can be obtained by agro-waste and bio-waste and this can be characterized in biogas, bio-ethers, solid biofuels, biofuels, bio-alcohol, biodiesel, and vegetable oils. They are classified into four generations, known as first generation, second generation, third generation, and fourth generation. By 2035, the global biofuel harvest is expected to meet 8% of the total demand for road transport fuel from the current level of 3% (Afolalu et. al., 2020).

In "**first-generation biofuel**", the main feedstocks are vegetable oils such as sunflower oil, soybean oil, mustard oil, olive oil, canola, etc. and harvesting foods such as corn, rice straw, sugarcane, and wheat, etc. Production of first-generation biofuel is not favorable because it directly competes with the supply of food. At present, commercialized biofuels are largely produced from first-generation biofuel feedstocks (Azad et. al., 2016). The usefulness of first-generation biofuels has been increasingly questioned due to concerns about competition for agricultural lands and raw materials (Abdullah et. al., 2019).

The "**second-generation biofuel**", is widely referred to as carbon neutral or carbon negative to its effect on carbon dioxide concentration (Joshi et. al., 2017). These biofuels are produced using biomass and have various advantages compared to the first-generation. This biomass can be introduced using small farms, as the whole plant can be used as raw substances. Further different types of crops can be grown together, which require less compost, and make raw materials more expensive (Bhatia et. al., 2017). Although this type of biofuel does not affect food supply, low yields and regional demand for the land area reduce their demand. Moreover, according to the wider culture of suitable plants, the first and second generations of biofuel have a significant impact on the environment (Farrokh et. al., 2019).

The "**third-generation biofuel**", namely algae biofuels, offers excellent opportunities. Algae can be grouped into two categories, namely macroalgae (known as seaweeds) and microalgae (Lee et. al., 2021). The production of biofuels from algal species, a few species of Chlorella species, and Scenedesmus species is a promising approach as algae grow rapidly, and compared to most of the world's terrestrial plants, it does not require soil and also has great potential to trap CO_2 and other depleting ozone gases (Satari et. al., 2019).

In the "**fourth-generation biofuel**", genetic modification has been performed on microorganisms, for example, microalgae, yeast, cyanobacteria, etc. for improving 4 photosynthetic efficiency, better light

penetration and reduction in photo-inhibition for higher levels of development, maximum oil content, and low structural complexity to get the numerous commercial applications. However, the 4th generation of biofuel is still in the early stages of formation, with potential natural and social hazards due to the chances for the destruction of genetically modified organisms in the climate (Kumar et. al., 2020).

Aquatic plants are invasive, floating plants that have serious ecological and economic disadvantages. They are found all over the world especially in tropical and subtropical areas. Because of their high level of development and the challenges of overcoming them, they cause serious damage to wetlands and also disrupt the water system, fish culture, hydropower generation, and drinking-water sources. They reduce daytime penetration into the water as they grow faster, cover all the water within a short time and reduce the amount of oxygen dispersed. The cost of water purification work is high, especially in removing reduced and new plants. It is important to find alternatives to reduce this (Arefin et. al., 2021). Aquatic biomass offer more profit than its terrestrial counterpart because it does not contradict food for land use. In addition, aquatic biomass has higher photosynthetic performance and a faster rate of growth compared to the lignocellulosic biomass (Wauton et. al., 2019).

Wastes of interest:

"**Water hyacinth**" (Eichhornia crassipes) is widely regarded as the world's worst, most enduring, insect-resistant, free-floating aquatic weed, because of its ability to cover all streams quickly (Dechassa et. al., 2020). Due to the high development rate (**220 kg/ha/day**), it adds a large amount of mat to the outer part of the water and severely degrades the marine environment. However, water hyacinth biomass is made primarily of high holocellulose (**44-66.9% dry matter**) with **low lignin content**. Hence, it can be regarded as a promising and cost-effective asset against food (Saratale et. al., 2020). WH can duplicate in 7 days with an annual efficiency elsewhere at a range of 930 and 2,900 tons per hectare (Ahou et. al., 2020). It has the following characteristics (Yigermal et. al., 2020).

- Seeds remain ready for significant extensions for up to 15 years.
- The average size of a water hyacinth is 40 cm but it can reach up to 1 m in height and has a high rate of vegetation multiplication and growth.
- The stems and leaves of water hyacinth contain air that provides the plant.

- Grows in mats up to 2 m thick and its plants are doubled in 15-18 days of asexual vegetative reproduction.

Petha wastewater (PWW)

Agra, the city of Taj is also well known for its delicacy, petha sweet produced from organic petha product (Benincasa hispida) and the existence of sweet petha industry goes back to the reign of the 16th century Mughal Emperor Jahangir. Petha cottage industries are located in the narrow streets of Noori Darwaja named after Queen Noor Jahan. As of now, about 600 petha industries are operating in Noori Darwaja with a daily output of 400 tons of petha sweet (Singhal et. al., 2015). During the production of petha, the large amount of organic solid and liquid waste separated resulting in land and water pollution respectively. The solid petha waste contains a fruit peel and seedy pulp. About 60% of organic petha fruit and about 40% of organic petha product is used for sweet preparation. Petha refinement involves the pretreatment of solid petha pieces with $Ca(OH)_2$ solution (approximately 50 kg of limestone used by each petha unit per day) which results in the solubilization of fruit carbohydrates in the dominant water known as PWW (Kumari et. al., 2020).

Hence it is proposed that water hyacinth would be the biomass used in the present research. Other waste used along with water hyacinth will be Petha wastewater.

Anaerobic Digestion (AD)

Anaerobic digestion is the technique which can solve the two crucial problems of waste disposal and energy crisis at the same time. It is the most effective treatment in which degradation of organic and inorganic components occur in the presence of molecular oxygen (Kumari et. al., 2019). It has several advantages for the treatment of organic solid wastes and pre-treatment strategies are compared in terms of different parameters like efficiency, energy balance, cost, and process sustainability (Bote et. al., 2020). It is easy to start just by using dung from ruminants which are easily available in rural areas and highenergy inputs are not required (Bhatia et. al., 2020). No contamination is provided by the anaerobic digestion strategy, as it plans to take advantage of the movement of bacteria that feed on the biomass substrate within the respondent (Carlini et. al., 2018). Usually, four important reactions occur during a complete anaerobic process forming methane: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. All of these reactions occur simultaneously and are independent (Kumari et. al., 2020).

Each of the reactions involved in the AD system occurs in the presence of a certain number of specific microbial species and the stability of the AD system critically depends on the establishment of this complex microbial community and composition. Consequently, it is necessary to identify the microbial composition and select corrective measures for the process in the digesters. However, while technical knowledge of the AD system in small and large-scale applications has been well established (Nie et. al., 2021).

- ✓ **Hydrolysis** (Chieng et. al., 2020): In the hydrolysis phase, macromolecules are broken down into specific particles such as basic sugars, amino acids, and unsaturated fats, which can be used by acidogenic bacteria.

$$nC_6H_{10}O_5 + nH_2O \rightarrow \ {}_nC_6H_{12}O_6$$

- ✓ **Acidogenesis** (Bajpai et. al., 2017): In acidogenesis, these components are fermented and converted into CO2, H2, natural acid, unsaturated fatty acids, and alcohol.

$$C_6H_{12}O_6 \rightarrow \ 2C_2H_5OH + 2CO_2$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH$$

- ✓ **Acetogenesis** (Biarnes et. al., 2013): This is followed by acetogenesis, in which unsaturated fatty acids and various intermediates are converted into acetic acid, CO_2, and H_2.

$$CH_3CH_2COOH + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3 + 3H_2$$

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 4H_2 + 2CO_2$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 3H_2 + H^+$$

- ✓ **Methanogenesis** (Kondusamy et. al., 2014): Acetic acid and hydrogen produced are converted into methane and carbon dioxide and hence, hydrogen is consumed.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Anaerobic digestion is mainly based on two important steps i.e.

- ❖ **Bio-hydrogen generation:** Biohydrogen is an energy carrier since its combustion generates entirely water and heat additionally encompasses a high energy yield of 7 122 kJ/g. traditionally gas is formed by chemical processes involving electrolysis of water and steam reforming. These processes

do not appear to be economically viable since it wants high energy input and high reaction temperature. Biohydrogen production is eco-friendly and may be created from a mixed or pure culture. Several anaerobic microorganisms can end up biohydrogen from organic wastes (Dwivedi. et. al., 2018).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$$

- **Methane formation or Consumption of hydrogen:** Methane is formed in the final reaction by methanogenic bacteria. These bacteria are capable of metabolizing methanol, CO_2, CO, formic acid, acetic acid, and hydrogen to methane (Bajpai et. al., 2017). Methanobrevibacter species, Methanobrevibacter formicicum, and Methanosarcina species are among the bacteria that synthesise methane. The amount of hydrogenotrophic species involved has a direct impact on the methane generation yield (Soeprijanto et. al., 2021).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Other biofuels like methanol and ethanol can be produced as:

- **Methanol production:** Methanol is an essential liquid fuel and a key component in the production of a variety of synthetic hydrocarbons. Methanol is used as a raw material in the petrochemical industry for the trans-esterification of numerous chemicals such as MTBE (Methyl tertiary-butyl ether), formaldehyde, acetic acid, and biodiesel. It is a high-octane liquid fuel that may be combined with gasoline and ethanol and used in today's vehicles with a relatively low cost. Today's methanol prices are comparable to those of hydrocarbon fuels (Kumari et. al., 2018).

$$CH_4 + 1/2H_2O \rightarrow CH_3OH$$

- **Ethanol production:** Ethanol has a much higher latent heat of vaporization (855 MJ/kg) than petrol (293 MJ/kg) as well as a higher-octane number (99) than petrol (80-100) and, as a result, pre-ignition does not occur when ethanol is used (Kumari et. al., 2014). Bioethanol replaces petroleum derivatives in energy production. It 8 may be combined with petroleum 4 in 5-10% blends known as E5, E10, etc., to promote the increase in its interests (Kumari et. al., 2019). India, a major sugar producer, has great potential to extract ethanol from its molasses; this makes ethanol generally well known among other biofuels in India. It is produced from either starch- or sugar- rich crops through

the process of fermentation. Following reaction takes place during the process (Gautam et. al., 2019).

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Saccharification and Fermentation: This process involves enzymatic production of ethanol in absence of oxygen using microbes such as Saccharomyces Cerevisiae (Kothari et. Al., 2012).

$$C_6H_{12}O_6 + 2H_2O \rightarrow C_2H_5OH + CH_3COOH + 2CO_2$$

LITERATURE SURVEY

Author	Title	Year	Conclusion
Alam et. al.	Aquatic weed as a biorefinery resource for biofuels and value-added products: Challenges and recent advancements	2021	Ethanol production from E. crassipes, observed 4.4 gL^{-1} upon fermenting the hydrolysate with Saccharomyces cerevisiae. When E. crassipes biomass was pretreated with acid-alkali followed by fermentation 0.21 g g^{-1} of ethanol production was observed.These studies showed that the same Eichhornia biomass showed varying yields by applying different pre-treatment methods and microorganisms. Thus, there is need to identify an efficient conversion technology and consortia of microorganisms for bio-ethanol production from aquatic weeds as the results are very promising.
Malode et. al.	Recent advances and viability in biofuel production	2021	The importance of biological resources for producing clean and renewable energy, as well as other renewable energy alternatives, is discussed in this article. The need for appropriate and renewable alternatives for fossil fuels are discussed.
Unpapom et. al.	Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer	2021	In this study, Anaerobic digestion methods produced biogas from crushed water hyacinth coupled with swine dung. AD was carried out for 45 days at mesophilic temperatures of 34-36 °C. Before batch dry AD, alkali (sodium hydroxide, NaOH) hydrolysis of water hyacinth was done at four distinct moderate concentrations. The biogas composition of CO_2 (29.08 %), CH_4 (68.89 %), and 1.09 % H_2S was highest when the optimal water hyacinth concentration was used in a 1:1 ratio of codigestion mixture with swine dung. CH_4 and CO_2 were found to be 92.35 % and 8.04 %, respectively, in upgraded biogas after chemical purification.
Abud et. al.	Ethanol from water hyacinth: A preliminary study of chemical pretreatment and enzymatic hydrolysis	2020	The complete parametric analysis involved in AD and alcohol fermentation explore that bioethanol generation from water hyacinth. Pre-formulated acid salts show a high fermentation rate. Enzymatic hydrolysis, acidic and alkaline is effective and most of the sugar has been melted and fermented.
Castro et. al.	Biomethanation of invasive water hyacinth	2020	AD water hyacinth has identified AD as an active, controlled, and enclosed version of the anaerobic

	from eutrophic waters as a post weed management practice in the Dominican Republic: a developing country		reduction of organic waste that releases methane. AD produces more than ten times the amount of energy consumed by mechanical harvesting. The differences in the characteristics of the water hyacinth in both water types were linked to the components of the water source.
Uche et. al.	Design and construction of fixed dome digester for biogas production using cow dung and water hyacinth	2020	Biofuel production from faecal of cow and water hyacinth biogas were studied using anaerobic biological modification. Performance tests were performed after biogas production after 21 days. The percentage of biogas produced indicates methane gas at 56.4%, carbon dioxide at 35% and nitrogen at 6.9%.
Barua et. al.	Biogas production from water hyacinth in a novel anaerobic digester: A continuous study	2019	The performance of the novel anaerobic digester's performance in distribution of the microorganisms, improving the digestibility of the feedstock, removing COD thereby enhancing the AD process is worth mentioning.
Wauton et. al.	Characterization of Water Hyacinth (Eichhornia Crassipes) For the Production of Thermochemical Fuels	2019	Water hyacinth can be better suited for pyrolysis and gasification reactions to produce bio-oil and bio-gas respectively, rather than biochar production. Water hyacinth can be heated directly to heat energy considering the maximum temperature value of 17.39 MJ / kg.
Ahmed et. al.	The Floating Aquatic Water Hyacinth (Eichhorniacrassipes) is a Multiuses Macrophyte for Fuel Ethanol Production and Pathogen control	2018	Water hyacinth is among the potential solutions to reduce the problems of climate changes and shortage of oil. It is a lignocellulosic biomass that can be utilized for reducing sugar production and thus further for bioethanol production. highest bioethanol yield of 4.1 g l^{-1} was produced by Saccharomyces cerevisiaeThermosacc and 0.10 M NaOH at 111°C for 120 min.
Madian et.al.	Bioethanol Production from Water Hyacinth Hydrolysate by Candida tropicalis Y-26	2018	About 14 g/l of bioethanol (185 mg/g of dry material) was produced by Candida tropicalis Y-26 using an aqueous production plant hyacinth hydrolyzate as the sole carbon source. A combination of Aspergillus terreus F-98 treatment with acid hydrolysis (H2SO4) to give a 409 mg/g amount of sugar reduction.
Dwivedi et. al	Water Hyacinth Feedstock: A Renewable Source for Bio-Ethanol Production	2018	In this study, a sample of water hyacinth was added using different alkalis (Sodium hydroxide and Potassium hydroxide) and acids (Hydrochloric acid, Sulfuric acid and Formic acid). Sulfuric acid treated with 4% v/v shows an excellent effect on blood sugar levels compared to other pretreatment chemicals that were 12.34 mg of sugar/g of Water hyacinth sample.
Priya et. al.	Biomethanation of water hyacinth biomass	2018	This paper explained, sun drying to increase water hyacinth biomass the solid content, preservation by insulation to ensure continuous water hyacinth biomass availability for AD and co-digestion with waste organic fractions proved to be effective for improving the net biogas yield as well methane content, making the bio-methanation of water hyacinth more feasible.

Kumari et. al	Pretreatment of lignocellulosic wastes for biofuel production: A critical review	2018	This paper review critically discusses and compares different pretreatment methods, biomass resources, the chemical composition of different agricultural biomass. Various pretreatment processes used for bio-hydrogen, bio-methane, bio-ethanol, bio-methanol bio-butanol, and bio-diesel production are also discussed.
Rezania et. al.	Review on Pretreatment Methods and Ethanol Production from Cellulosic Water Hyacinth	2017	This review presents the recent studies in pretreatment, hydrolysis, and fermentation of water hyacinth biomass into ethanol. Based on the literature review in the case of water hyacinth, the most frequently used pretreatment methods were acid and alkali, while ionic liquid and microwave-assisted methods were used rarely. The dominant sugars were glucose, xylose, galactose, arabinose, and mannose. Based on the findings, cellulase and S. cerevisiae were mostly used for enzymatic hydrolysis and fermentation of water hyacinth to ethanol, respectively.
Sindhu et. al.	Water hyacinth a potential source for value addition: an overview	2017	This review discusses, the recent research and developmental activities on the bioconversion of water hyacinth for the production of fuels and value-added products which helps in the selfsustainability especially for developing countries as well as its possibilities and challenges in commercialization.
Azad et. al.	A review on socio-economic aspects of sustainable biofuels	2016	The 2nd generation biofuels are sustainable alternative fuels that have more potential to provide favorable economic outcomes than 1st generation biofuels in Australia. There is a need for further studies in the area of emission reduction and economic production technology for 2nd generation biofuels for commercial application.
Zhang et. al.	Optimization of Bioethanol Production Using Whole Plant of Water Hyacinth as Substrate in Simultaneous Saccharification and Fermentation Process	2016	As a conventional invasive weed, water hyacinth proved its feasibility for bioethanol energy production. The experiment, 402.93 mg reducing sugar and 1.289 g/L bioethanol were achieved using water hyacinth substrate in a Simultaneous SSF process after being optimized by response surface methodology. This relatively high bioethanol production indicates that water hyacinth is a promising plant in the research and development of sustainable energy
Cheng et. al.	Concurrent calcium peroxide pretreatment and wet storage of water hyacinth for fermentable sugar production	2015	This study discovered that the concurrent pretreatment and wet storage of fresh aquatic biomass could be achieved simply by using CaO_2. It suggested that at least 10% CaO_2 loading and 80% moisture content are required to maintain the alkali pH and obtain good solid recovery over 90-day storage. The best results of lignin removal, carbohydrate preservation and enzymatic saccharification obtained in this work were comparable to those in other reports.
Kumari et. al.	Bioethanol production from water hyacinth biomass using isolated	2014	During the enzymatic saccharification process, the maximum yield of reducing sugar and xylose was found to be 21.08mg/g and 236.56 mg/g

	fungal strain from local environment		respectively. Also, it was demonstrated that the hydrolysate of WHB can be fermented using Pichia stipitis which resulted in an ethanol concentration of 3.913 g/L. This was because it can degrade pentose in addition to hexoses unlike others.
Singh et. al.	Comparative study of various pretreatment techniques for ethanol production from water hyacinth	2013	Pretreated water hyacinth was used as substrate for enzyme production by Aspergillus niger and onsite produced enzymes were used for saccharification. The enzymatic hydrolysate was concentrated and fermented by Saccharomyces cerevisiae, Scheffersomyces stipitis and by co-culture of both, with ethanol concentrations 4.3 g/L, 6.2 g/L and 9.8 g/L, respectively. During co-culture, ethanol production was 56% high than S. cerevisiae alone and 36% high than S. stipitis alone.
Ganguly et. al.	Studies on ethanol production from water hyacinthA revie	2012	The method described above reduces plant population while providing a simple and low-cost procedure ideal for developing countries, in which the cellulose parts of the herbs are hydrolysed by enzymes into glucose sugar, which is then fermented to bioethanol. It has been discovered that 1 kg of cellulose yields 1.1 kg of glucose and 0.56 kg of ethanol. The hemicellulose sugars are also fermented to produce bioethanol.

OBJECTIVES

✓ Pretreatment of water hyacinth with alkaline waste such as PWW under different conditions would be carried out.

✓ The effect of the above pretreatment methods for the optimum production of biofuels e.g., bio-methanol and bioethanol from water hyacinth would be studied.

✓ The combination of results obtained will be studied using available statistical/mathematical tools.

✓ Correlation of results obtained would be evaluated.

Based on literatue review and pilot study, the targeted production of biofuel would be **3 g/L-7 g/L** using water hyacinth with alkaline waste.

METHODOLOGY

Reported pretreatment methods are either, physical, chemical, or enzymatic. An attempt will be made to pretreat the lignocellulosic waste (water hyacinth) with another waste (alkaline in nature) instead of using chemicals. This pretreatment will turn waste hydrolysis into simple sugars which can be fermented into biofuels with the help of anaerobic microbes.

- For enhanced biofuels production from aquatic wastes, pretreatment of the water hyacinth with PWW (an alkaline waste) would be carried out.
- Biofuels produced would be quantified using Gas Chromatography.
- Initial and final (at the end of the process) surface analysis of the water hyacinth would be done by different techniques like FESEM, FTIR, XRD, etc.
- Optimization of the process will also be done to get a better production yield.
- In addition to this progress of the experiment will also be monitored using various physical parameters like glucose, chemical oxygen demand (COD), and total solids (TS).

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